## REMARKS

Claims 1, 3-15 and 17-21 are pending in the present application. Claims 11-15 and 19 have been withdrawn. Claim 1 is in independent form. Claim 18 is amended. In view of the above amendments and following remarks, favorable reconsideration and allowance of the present application is respectfully requested.

### I. 35 U.S.C. §112, Second Paragraph Rejection

Claim 18 stands rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants claim as the invention.

Namely, the rejection states "[i]t is unclear how target sequences can be immobilized in the analyte solution, since immobilization would require that the target sequences are no longer in solution." Action, p. 2.

By the present Amendment, Applicants submit that claim 18 has been amended to remove the term "immobilized."

Thus, Applicants submit that the §112, second paragraph rejection has been overcome. Accordingly, reconsideration and withdrawal is respectfully requested.

### II. Example Embodiments

Example embodiments teach that the proposed method permits a larger variety of different possible designs in the case of simultaneous or multiplex studies. The reason for this is, inter alia, that it is not necessary

to incorporate a label into amplicons produced during the PCR, which, especially in complex tests, holds the risk of undesired interactions arising between the substances required for labeling and between these and target sequences to be identified. See paragraph [0022] of the published application.

It is explicitly explained in the present application that no labels or markers must be used to detect the DNA through impedance measurements It is the high concentration of DNA created by the PCR of the numerous "target molecules" that are three-dimensionally bound in the gel by the numerous "probe molecules," which makes possible the electro-chemical detection via impedance measurements.

III. CITED ART GROUNDS OF REJECTION

*(A) Claims 1, 3, 4, 7, 8, 17, 20 and 21 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Cheng et al. (hereinafter "Cheng"), U.S. Patent Publication No. 2002/0155586 A1 in view of Frechet et al. (hereinafter "Frechet"), U.S. Publication No. 2004/0101442 A1 and further in view of Hodko et al. (hereinafter "Hodko"), Detection of Pathogens Using On-Chip Electrochemical Analysis of PC Amplified DNA Molecules, Proceedings of SPIE, Vol. 4265, p. 65-74 (2001). Applicants respectfully traverse the rejection.*

i. INDEPENDENT CLAIM 1

Independent claim 1 is directed to a method for PCR amplification and detection of nucleotide sequences including (*inter alia*) "using a hydrophilic reaction layer having coupling groups for covalent binding of probe molecules and an array of a plurality of microspots forming analytical positions" and "detecting hybridization events on the probe molecules immobilized at one of the analytical positions electrochemically with the aid of a microelectrode arrangement wherein detected nucleotide sequences alter impedance of the microelectrode arrangement." Applicants submit that the combination of Cheng, Frechet and Hodko fails to explicitly teach, or otherwise suggest, the above features recited in independent claim 1.

a. CHENG, FRECHET AND HODKO

The rejection states that "Cheng does not teach a method for PCR amplification and detection of nucleotide sequences using a hydrophilic reaction layer having coupling groups for covalent binding of probe molecules..." Action, p. 5. Thus, the Examiner relies on Frechet, and states that "Frechet teaches methods of grafting polymer monolith surfaces for microfluidic devices such as a 'lab on a chip' that includes attachment of polymer chains having functional groups..." Action, p. 6.

The rejection further states that it would have been "...obvious to one having ordinary skill in the art at the time of the invention was made to combine the methods of Cheng for making and using an integrated system such as a chip for amplification and detection of nucleic acid targets directly

on the chip device with the methods of Frechet for making similar lab-on-a-chip devices that can contain any of a variety polymers grafted on the surface that have functional groups attached that would be useful for covalent attachment of the oligonucleotide capture probes taught by Chen..." Action, p. 6-7.

Firstly, Frechet is directed to "...a microfluidic device formed from a surface-modified rigid substrate such as a thermoplastic polymer, having a channel containing a porous polymer monolith." Frechet, paragraph [0014]. Frechet teaches that,

> ...almost all of today's reported microfluidic chips feature open channel architecture. Hence, the surface to volume ratio of these channels is rather small. This is a serious problem in applications such as chromatographic separations, heterogeneous catalysis, and solid phase extraction that rely on interactions with a solid surface. Since only the channel walls are used for the desired interaction, these microdevices can handle only minute amounts of compounds.
>
> Frechet, paragraph [0009],

Thus, Frechet teaches that microfluidic devices rely on solid surface interaction between the fluid passing through the device and the channel walls of the device (*i.e.*, fluidics).

Cheng acknowledges the conventional total chemical analysis system (TAS) (as well as other conventional systems), but states that "[d]espite the long-recognized need for such an integrated system without a complicated fluidics and inadequate valve systems, no satisfactory solution has previously been proposed." Cheng, paragraph [0020]. Thus, Applicants

submit that Cheng "teaches away" from the complicated fluidics of Frechet's total chemical analysis system.

Applicants respectfully remind the Examiner of MPEP §2141.02 (VI), which states that "[a] prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away form the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F. 2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984)..."

Secondly, Cheng is directed to a method for multi-step sample preparation and molecular diagnostic analysis of biological materials wherein,

> The sample may be introduced into the flow cell via an input port coupled to the flow cell. A dielectric force pattern may be created by individually biasing the electrodes positioned within the flow cell. The sample may then be subjected to the dielectric force pattern in order to separate the undesired cells from the desired cells in the sample. Then, the desired cells may be isolated by maintaining an attractive bias for the desired cells and introducing a flow of wash buffer through the flow cell via the input port to eliminate the undesired cells.
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> Cheng, paragraph [0025].

Thus, Cheng teaches that desired cells are separated from the undesired cells by maintaining an attractive bias for the desired cells.

On the contrary, Frechet teaches that desired analytes are separated from the undesired analytes by coupling the desired analytes to the functional groups of a polymer monolith attached to the surface of a microfluidic device.

Therefore, Applicants submit that, to combine the polymer monolith surface of Frechet with the flow cell shown in Fig. 5 of Cheng (as asserted by

the Examiner), would change the principle of operation of Cheng. That is, the desired cells would be separated by coupling to the functional groups of a polymer monolith attached to the surface of the substrate 10, not by maintaining an attractive bias for the desired cells (as taught by Cheng).

Applicants remind the Examiner that "[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959)" MPEP 2143.01(VI) (emphasis added).

Thirdly, referring to Fig. 5, Cheng teaches that the microchip having the microarray 23 is in a chamber, which is used for realizing electro-chemical measurements.

On the other hand, Frechet teaches a microfluidic device is provided with channels for mixing liquids. Applicants can find no teaching in Frechet that suggests the microfluidic device has a chamber, a micro-spot array, or electro-chemical electrodes. Frechet does not provide any teaching regarding how an array of spots with "probe molecules" could be generated in the channel with a hydrogel. Thus, Applicants submit that one of ordinary skill in the art would not look to Frechet to cure the deficiencies of Cheng.

Accordingly, there is no motivation to combine Frechet and Cheng, absent inappropriate hindsight of the Applicants' own disclosure.

Fourth, Cheng only mentions a glass substrate ("...a glass slide-based device having exposed, i.e., naked, interdigitated electrodes plated on the surface of the slide..." Cheng, paragraph [0006]).

However, Frechet teaches that the substrate is plastic, and advantageous over the conventional glass substrate because "...the cost of the multistep wet fabrication of these microfluidic chips [with inorganic substrates] is high and the use of thermoplastic polymer materials instead of hard inorganics would enable the use of inexpensive 'dry' techniques such as injection molding or hot embossing." Frechet, paragraph [0007] (emphasis added).

For at least these reasons, Applicants submit that there is no motivation to combine the teachings of Frechet and with Cheng. Not only is there no motivation to combine Cheng and Frechet in the alleged manner, but the combination of Cheng and Frechet would not lead to the method recited independent claim 1.

The rejection further states that "Cheng also does not teach a method wherein detected nucleotide sequences alter impedance of the microelectrode arrangement." Action, p. 6. Thus, the Examiner relies on Hodko, and states that "Hodko teaches a method for detection of pathogens based on electrochemical detection of PCR amplified molecules specific for pathogens wherein the detection method is based one electrochemical AC impedance analysis using redox probes capable of intercalating into double-stranded DNA products in contact with platinum electrodes (p. 66, lines 16-19 and p. 68, lines 1-12). Action, p. 6. Further,

> ...the formation of double-stranded products at the electrode surface can be detected using the electrochemical AC impedance method of Hodko since this method 'is easily adapted to a microfluidic environment' (Hodko, p. 66, lines 16-18), providing direct detection of PCR amplicons within a thin microfluidic chamber with no need for modifying the electrodes (Hodko, p. 66, lines 30-32 and p. 72, lines 24-26), without interference from PCR reagents present in the sample (Hodko, p. 72, line 26).
>
> Action, p. 7-8.

Firstly, Hodko is directed to a microfluidics based system that utilizes electrochemical detection of the PCR amplified DNA molecules specific for a targeted pathogen wherein three individual electrodes (a reference electrode, a counter electrode and a working electrode) are used to detect ds-DNA molecules with the aid of impedance measurements. The three-electrode configuration of Hodko in no way corresponds to the working electrode configuration of Hodko or the " microelectrode arrangement" recited in claim 1.

Secondly, Hodko teaches that "[a]n impedance spectroscopy based method is used to detect DNA amplicons in the presence of DNA intercalating redox probe, which further amplifies the detection signal." Hodko, *Abstract*. In particular, Hodko teaches that "[i]n the presence of redox mediators capable of intercalating into the DNA molecule, this signal is electrochemically amplified. A number of redox-probes were tested to determine which of the probes provide the largest signal in the presence of DNA." Hodko, p.68. Thus, Hodko teaches ds-DNA molecules are detected using impedance measurements after intercalation with markers. In other words, the ds-DNA is bound by markers, then the signal of the markers is

detected. Thus, the detected DNA does not "alter impedance of the microelectrode arrangement" as recited in claim 1.

Thirdly, even assuming *arguendo* that one of ordinary skill were motivated to combine the teachings of Hodko and Cheng (which Applicants do not agree with), Applicants submit that one would use markers as taught by Hodko in place of the permeation layer of Cheng. Thus, the resulting device would not have "a hydrophilic reaction layer" as recited in claim 1. Therefore, Applicants submit that the use of markers "teaches away" from the three-dimensional binding of the target molecules in "a hydrophilic reaction layer" as recited in claim 1.

As such, Hodko also fails to cure the deficiencies of Cheng with respect to independent claim 1.

For at least these reasons, Applicant submit that Cheng in view of Frechet and further in view of Hodko fails to explicitly teach, or otherwise suggest, a method for PCR amplification and detection of nucleotide sequences including "using a hydrophilic reaction layer having coupling groups for covalent binding of probe molecules and an array of a plurality of microspots forming analytical positions" and "detecting hybridization events on the probe molecules immobilized at one of the analytical positions electrochemically with the aid of a microelectrode arrangement wherein detected nucleotide sequences alter impedance of the microelectrode arrangement" as recited in independent claim 1.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection to independent claim 1, and claims 3,

4, 7, 8, 17, 20 and 21 at least by virtue of their dependency on independent claim 1.

ii. CLAIM 4

Claim 4 is directed to a method for PCR amplification and detection of nucleotide sequences including (*inter alia*) "a free-radically crosslinkable hydrogel based on at least one of acrylamide with maleic anhydride and glycidyl (meth)acrylate as coupling groups is used." Applicants submit that the combination of Cheng, Frechet and Hodko fails to explicitly teach, or otherwise suggest, the above features recited in claim 4.

a. THE COMBINATION OF CHENG, FRECHET AND HODKO

The rejection state that Cheng does not teach "...a cross-linkable hydrogel based on acrylamide with either maleic anhydride or glycidyl (meth)acrylate as coupling groups." Action, p. 5. Thus, the Examiner relies on Frechet, and states that "Frechet teaches methods of grafting polymer monolith surfaces for microfluidic devices such as a 'lab on a chip' that includes attachment of polymer chains having functional groups such as hydrophilic or reactive groups comprising acrylamide that bears functional groups including glycidyl methacrylate (paragraph 6, lines 1-17, paragraph 27, lines 1-10, paragraph 28, lines 1-3 and paragraph 29, lines 1-7)." Action, p. 6.

Firstly, Frechet teaches a channel coated with a porous monolith that results from UV supported deposition. Frechet fails to teach a "crosslinkable hydrogel based on acrylamide" as asserted by the Examiner.

Secondly, a porous monolith coating cannot be transferred without problems to a flat array of electrodes. Namely, the electrodes of Cheng are metal, whereas the substrate of Frechet is plastic. Furthermore, deposition inside a channel occurs differently than on a flat surface. As such, it would be difficult to produce a closed flat gel layer using the method taught by Frechet.

For at least these reasons, Applicant submit that Cheng in view of Frechet and further in view of Hodko fails to explicitly teach, or otherwise suggest, a method for PCR amplification and detection of nucleotide sequences including "a free-radically crosslinkable hydrogel based on at least one of acrylamide with maleic anhydride and glycidyl (meth)acrylate as coupling groups is used" as recited in claim 4.

Accordingly, Applicants respectfully submit that claim 4 is patentable by virtue of its dependency on independent 1, as well as for its own merits. Thus, reconsideration and withdrawal of the rejection to claim 4 is respectfully requested.

*(B) Claims 5 and 6 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Cheng in view of Frechet and Hodko and further in view of Ghodsian, U.S. Publication No. 2002/0115293 A1. Applicants respectfully traverse the rejection.*

Ghodsian is directed to a chip device for sequencing long DNA fragments using optical detection. Ghodsian fails to teach, or suggest, electrochemical detection using a microelectrode arrangement wherein detected nucleotide sequences alter impedance of the microelectrode arrangement. Thus, Ghodsian fails to cure the deficiencies of Cheng, Frechet and Hodko with respect to independent claim 1.

Applicants submit that claims 5 and 6, at least by virtue of their dependency on independent claim 1, are patentable over the combination of Cheng, Frechet, Hodko and Ghodsian.

As such, Applicants respectfully request that the Examiner reconsider and withdraw the rejection to claims 5 and 6.

*(C) Claims 9, 10 and 18 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Cheng in view of Frechet and Hodko and further in view of Strizhkov et al. (hereinafter "Strizhkov"), PCR Amplification on a Microarray of Gel-Immobilized Oligonucleotides: Detection of Bacterial Toxin- and Drug-Resistant Genes and Their Mutations, BioTechniques, 29(4):844-846, 848, 850-852, 854, 856-857 (Oct. 2000). Applicants respectfully traverse the rejection.*

Strizhkov, directed to PCR amplification, teaches that "[t]he kinetics of amplification was measured in real time in parallel for all gel pads with a fluorescent microscope equipped with a charge-coupled device (CCD) camera." Strizhkov, Abstract. Thus, Strizhkov teaches using optical detection, not electrochemical detection using a microelectrode arrangement

wherein detected nucleotide sequences alter impedance of the microelectrode arrangement. Thus, Strizhkov fails to cure the above-noted deficiencies of Cheng, Frechet and Hodko with respect to independent claim 1.

Applicants submit that claims 9, 10 and 18, at least by virtue of their dependency on independent claim 1, are patentable over the combination of Cheng, Frechet, Hodko and Strizhkov.

As such, Applicants respectfully request that the Examiner reconsider and withdraw the rejection to claims 9, 10 and 18.

## IV. REQUEST FOR REJOINDER

In the event that independent claim 1 is held allowable, Applicants respectfully request rejoinder of withdrawn claims 11-15 and 19, which depend from and therefore require all of the features of claim 1.

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**CONCLUSION**

Accordingly, in view of the above, reconsideration of the rejections and allowance of each of claims 1, 3-15 and 17-21 in connection with the present application is earnestly solicited.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 08-0750 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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